Oecologia (Berlin) (1985) 67:205–208

*Oecologia*


# The use of stable carbon isotope analysis in rooting studies

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**Summary.** Stable carbon isotope analysis was evaluated as a means of predicting the relative proportions of $C_3$ and $C_4$ root phytomass in species mixtures. The following mixtures of $C_3$ and $C_4$ species were used: 1) big bluestem (*Andropogon gerardii*)/cheatgrass (*Bromus tectorum*), 2) little bluestem (*Schizachyrium scoparium*)/cheatgrass, and 3) sorghum (*Sorghum bicolor*)/sunflower (*Helianthus annuus*). There was a significant correlation ($P<0.01$) between % $C_4$ phytomass and stable carbon isotope values for each of the three combinations ($r^2>0.98$). Root length per mass varied among the five species studied (10.1–94.3 m/g), which resulted in different conclusions depending on whether root values are expressed as length or mass. For example, field samples from a tallgrass prairie site were estimated to contain about 20% cheatgrass on a mass basis, whereas the figure was 68% when expressed in terms of length. The combination of stable carbon isotope analysis with length-for-mass measurements promises to be a useful means of studying root competition between $C_3$ and $C_4$ plants.

Root systems of plants occupying similar aboveground locations may compete (Remison and Snaydon 1980; Bookman and Mack 1982), or they may reduce or avoid competition by occupying different depths within the soil profile (Fitter and Hay 1981). However, due to methodological problems, root competition has seldom been assessed, and root systems of plant communities are generally dealt with as a single unit rather than on a species basis (Lorenz 1977). Methods for studying roots tend to be time-consuming, difficult, and often inaccurate (Bohm 1979). As a result, assessment of belowground competition has seldom been attempted.

Stable carbon isotope analysis has been suggested as a method for determining the proportion of cool season ($C_3$) and warm season ($C_4$) species in mixed tissue samples of aboveground biomass, roots, seeds, and herbivore diets (Ludlow et al. 1976). The basis of this procedure is that $C_3$ species discriminate against $^{13}CO_2$ during photosynthesis to a greater extent than do $C_4$ species (O'Leary 1981). As a result of this fractionation during photosynthesis, $C_3$ plants contain approximately 14 parts per thousand less carbon-13 than $C_4$ plants. $C_3$ plants have $\delta^{13}C$ values ranging from $-22$ to $-31‰$ (mean $=-26‰$), while $C_4$ plants typically range from $-9$ to $-15‰$ (mean $=-12‰$).

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This natural carbon isotope label has been exploited to trace a variety of ecological processes. For example, the stable carbon isotope technique has been applied to studies of diet selection by rangeland insects (Boutton et al. 1980, 1983a), and several species of larger herbivores (Jones et al. 1979; Tieszen et al. 1979). However, there are no studies confirming the value of this technique in studying rooting mixtures. The objectives of this study were to: 1) evaluate stable carbon isotope analysis as a method of predicting relative proportions of $C_3$ and $C_4$ biomass in mixtures of root material, and 2) determine the relationships between phytomass and root length proportions.

## Materials and methods

All samples were collected at the USDA/ARS Southwestern Livestock and Forage Research Station (98°0′W, 35°40′N; elevation = 450 m) near El Reno, Oklahoma, USA. Root samples of big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), and cheatgrass (*Bromus tectorum* L.) were collected from a tallgrass prairie site on 16 May, 1983. Root samples of sunflower (*Helianthus annuus* L.) and sorghum (*Sorghum bicolor* (L.) Moench) were collected from a sorghum field on 26 September, 1983. To insure proper species identification, only surface roots (0–10 cm depth) which were attached to aboveground plant parts were collected. Samples were collected from 10 randomly located plots on the tallgrass prairie, and from five individual plants of each species in the sorghum field. In addition, nine root cores measuring 7.6 cm in diameter were taken to a depth of 30 cm on the tallgrass prairie site. The root cores were taken below plots which contained only the two bluestems and cheatgrass in a $30 \times 30$ cm area.

Roots were thoroughly washed to remove adhering soil particles, and soaked in 0.8 N HCl for approximately 1 h to remove any exogenous carbonates (which tend to be relatively enriched in $^{13}C$) from the root surfaces. Five mixtures of each of the following $C_3/C_4$ species pairs were created from the processed roots: 1) big bluestem ($C_4$)/cheatgrass ($C_3$), 2) little bluestem ($C_4$)/cheatgrass ($C_3$), and 3) sorghum ($C_4$)/sunflower ($C_3$). These mixtures ranged from 0 to 100% $C_4$ phytomass. Before the $C_3$ and $C_4$ components were mixed, each component was scanned for root length (Comair Rootlength Scanner, Commonwealth Aircraft Corp., Melbourne, Australia)[1], dried for 72 h at 60 C

[1] Use of company names does not imply preferential treatment or endorsement by USDA

and weighed. The components of each mixture were then combined and ground twice in a Cyclone Sample Mill (U-D Corp., Boulder, CO). Samples of individual species and root core samples were also ground in this manner. Before grinding, all non-fibrous roots were removed from the root core samples to insure that dormant or lateral growing forb roots did not interfere with the analysis. Because ash values were low (8–10%) and varied little between species, mass was not expressed on an ash-free basis.

Root samples were combusted to carbon dioxide in sealed pyrex tubes according to the procedure outlined by Boutton et al. (1983b). Approximately 5 mg of dried sample, 0.5 g of copper oxide wire, and 9 $mm^2$ of silver foil were placed in a 20 cm length of pre-combusted 9 mm OD pyrex tubing previously sealed at one end. Sample tubes were then attached to a vacuum line, evacuated to $7 \times 10^{-3}$ torr, and sealed with a torch. The sealed tubes were placed in a muffle furnace at 550° C for 1 h.

Gases from the combusted tubes were released into a vacuum line where the $CO_2$ was cryogenically purified and transferred into 20 cc Vacutainers® (Becton-Dickinson, Rutherford NJ) for mass spectrometric analysis on an automated Nuclide 3–60 SecTorr isotope ratio mass spectrometer (Schoeller and Klein 1979). Stable carbon isotope ratios were determined and correction factors applied according to Craig (1957), and the results are expressed as:

$$\delta^{13}C‰ = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 10^3$$

where $R$ is the mass 45 to 44 ratio. All results are reported relative to the international PDB standard. The standard deviation for replicate combustions of the same sample was 0.34‰. Repeated measurements of a single gas yielded a machine error of 0.10‰.

## Results and discussion

There was a significant correlation ($P < 0.01$) between $\delta^{13}C$ and the % of $C_4$ phytomass for all three species mixtures (Figs. 1–3). In all three cases, stable carbon isotope ratios were able to account for at least 98% of the variation in the $C_4$ composition of the root mixtures. The regression equations were nearly identical for the two bluestem/cheatgrass combinations (Figs. 1 and 2). The slope of the sorghum/sunflower regression line (Fig. 3) was somewhat steeper given that sorghum had $\delta^{13}C$ values more negative (−13.2) than either big bluestem (−11.7) or little bluestem (−11.2). The two $C_3$ species had similar $\delta^{13}C$ values: −26.1 and −26.3 for cheatgrass and sunflower, respectively. These data suggest the regression relationships may be similar between $C_3/C_4$ combinations in which individual species have comparable $\delta^{13}C$ values. Because small seasonal differences in the $\delta^{13}C$ values of plant parts have been demonstrated (Lowdon and Dyck 1974), it is recommended that predictive equations be checked each time sampling occurs in temporal studies. These small (0.2–5.0‰) seasonal variations are due to changes in environmental conditions as the growing season progresses (Smith et al. 1976; Bender and Berge 1979).

These results indicate that stable carbon isotope analysis can provide an estimate of the composition of mixed $C_3$ and $C_4$ root samples, as Ludlow et al. (1976) previously suggested. The proportions of $C_3$ and $C_4$ species must initially be determined on a weight basis, since stable carbon isotope analysis is directly influenced by relative mass of each component. However, in rooting studies, length is per-

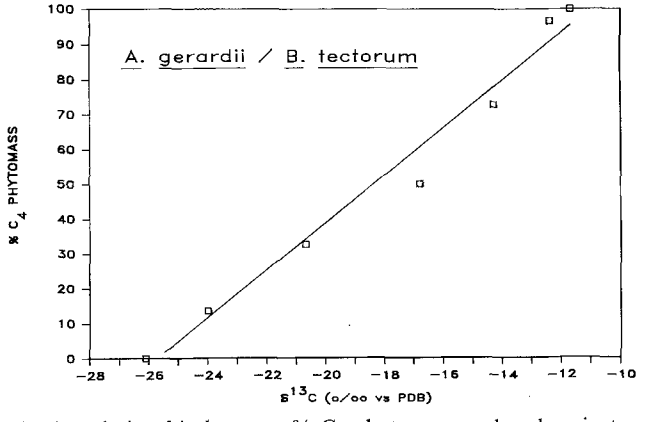


**Fig. 1.** Relationship between % $C_4$ phytomass and carbon isotope ratio for *Andropogon gerardii/Bromus tectorum* root mixtures. The equation for this relationship is $Y = 175.0 + 6.8(X)$, $r^2 = 0.978$, and the standard error of the estimate = 2.9

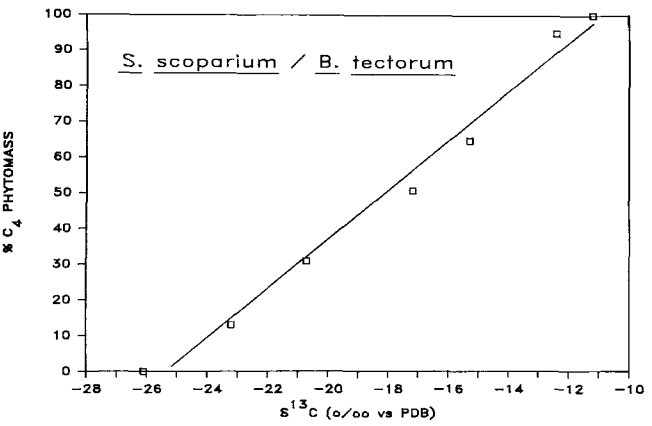


**Fig. 2.** Relationship between % $C_4$ phytomass and carbon isotope ratio for *Schizachyrium scoparium/Bromus tectorum* root mixtures. The equation for this relationship is $Y = 175.0 + 6.9(X)$, $r^2 = 0.986$, and the standard error of the estimate = 2.2

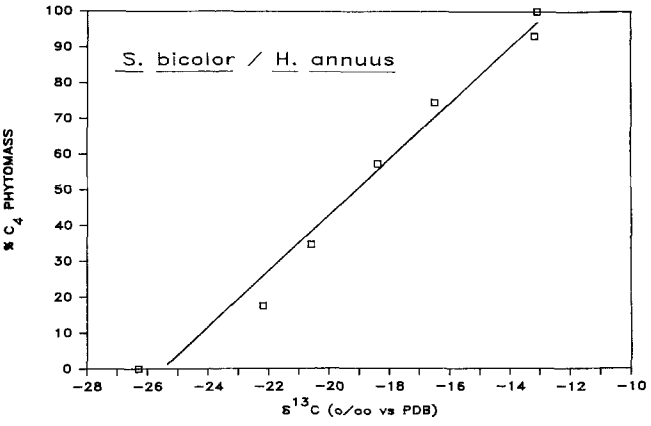


**Fig. 3.** Relationship between % $C_4$ phytomass and carbon isotope ratio for *Sorghum bicolor/Helianthus annuus* root mixtures. The equation for this relationship is $Y = 199.1 + 7.8(X)$, $r^2 = 0.982$, and the standard error of the estimate = 2.5

**Table 1.** Root length per mass for a range of species and growth forms

| Species | Growth form | Root length per mass (m/g) | Source |
|---|---|---|---|
| *Andropogon gerardii* | perennial grass | 10.1 | Present study |
| *Schizachyrium scoparium* | perennial grass | 12.6 | Present study |
| *Bromus tectorum* | annual grass | 94.3 | Present study |
| *Helianthus annuus* | annual forb | 42.0 | Present study |
| *Sorghum bicolor* | annual cereal | 23.2 | Present study |
| *Triticum aestivum* | annual cereal | 240.9 | Gregory et al. (1978)[a] |
| *Zea maize* | annual cereal | 141.0–290.0 | Neilsen and Barber (1978)[b] |
| *Carex aquatilis* | perennial sedge | 6.3 | Chapin and Slack (1979)[c] |
| *Eriophorum vaginatum* | perennial sedge | 25.0 | Chapin and Slack (1979)[c] |
| *Arctostaphylos pungens* | shrub | 8.6– 11.2 | Kummerow (1980)[d] |
| *Arctostaphylos glauca* | shrub | 7.1– 8.7 | Kummerow (1980)[d] |
| *Yucca whipplei* | shrub | 3.5– 4.8 | Kummerow (1980)[d] |
| *Adenostoma fasciculatum* | shrub | 20.5– 23.9 | Kummerow (1980)[d] |

[a] We used the regression equation for root length vs. root dry weight to obtain an average m/g value
[b] Range for hydroponically grown seedlings of 12 diverse genotypes
[c] For undefoliated plants
[d] Values for fine roots of 1.5 year old seedlings grown under several water regimes

haps more important from a functional point of view, because length is thought to be more highly correlated with water and nutrient absorption (Newman 1966; Bohm 1979). Root length per mass for the species we studied and some literature values appear in Table 1. The values reveal the species-specific (and in one case genotype specific) nature of this parameter. In some instances relative root phytomass values are sufficient, but in general determining root length will aid in interpretation of data, particularly in studies of species mixtures.

Data from the root cores can be used to illustrate the comparison between root mass and root length in a mixed sample. The nine root core samples taken beneath bluestem/cheatgrass plots yielded an average $\delta^{13}C$ value of $-14.0 \pm 1.7$ (1 standard deviation). The regression model from either Fig. 1 or 2 would predict that the samples contained approximately 80% $C_4$ phytomass. However, the relative $C_3/C_4$ composition proved to be quite different when expressed on the basis of length. If we consider a one gram sample and average the root length per mass (Table 1) of the two bluestems (both were present in our root cores), the following comparison can be made: 0.2 g ($C_3$ phytomass) $\times 94.3$ m/g $= 18.9$ m of cheatgrass root; 0.8 g ($C_4$ phytomass) $\times 11.4$ m/g $= 9.1$ m of bluestem roots. In this example cheatgrass constituted only 20% of the mass of the sample, but 68% of the total length. If length is indeed more important than mass from a functional point of view, then the importance of determining length/mass in such studies is readily apparent.

It should be noted that surface roots were sampled to establish the root length per mass relationship. Our experience suggests that root length per mass will vary with depth in the soil profile. The bluestems appeared to have thicker roots at the surface, and our root length per mass values are probably lower than if we had excavated roots to the 30 cm depth. In studies where root mass is to be converted to rooth length, we recommend establishing the length/weight relationship at each of the depths to be sampled.

There are a number of instances in which stable carbon isotope analysis may aid in the study of root competition. Ludlow et al. (1976) have suggested that the technique may be useful in grass-legume mixes, where the grass is $C_4$ and the legume $C_3$. Many improved pastures in tropical and subtropical environments have this combination of a tropical grass ($C_4$) and a legume ($C_3$). $C_3$ and $C_4$ species also coexist in many other grassland and desert environments throughout the world. For example, in the Great Plains of North America, cool season grasses are typically $C_3$ species, while warm season grasses are generally $C_4$. In this particular case, stable isotope techniques would permit studies on the root systems of physiologically and ecologically distinct components of the grassland. Finally, weedy species often possess a photosynthetic pathway different from that of the crop with which they compete. In their assessment of weed infestation in crops, Holm et al. (1977) indicate that $C_4$ species tend to predominate among the "world's worst weeds". Since most crops are $C_3$ species, stable isotope analysis should be useful in weed/crop root competition studies.

*Acknowledgements.* Portions of this research were supported by NIH grant AM28129. The authors thank Jim Browning, Mary Nelson, and Michelle Steinfeld for technical assistance; Sheri Wahl for manuscript preparation; and Paul Doescher, David Forbes, and William W. Wong for reviewing the manuscript.

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Received February 14, 1985